Applications of Elemental Bio-Imaging and Development of Novel Quantification Methods

by

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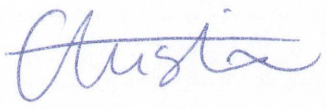
Certificate of authorship and originality

I certify that the work in this thesis has not previously been submitted for a degree nor has it been submitted as part of the requirements for a degree except as fully acknowledged within the text.

I also certify that the thesis has been written by me. Any help that I have received in my research work and the preparation of the thesis itself has been acknowledged. In addition, I certify that all the information sources and literature used are indicated in the thesis.



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29/07/2011

DATE

A little learning is a dang'rous thing;

Drink deep, or taste not the Pierian Spring:

There shallow draughts intoxicate the brain,

And drinking largely sobers us again.

-Alexander Pope, *An Essay on Criticism* (1711)

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1. Austin C., D. Hare, A.L. Rozelle, W.H. Robinson, R. Grimm and P. Doble, *Metallomics*, Elemental bio-imaging of calcium phosphate based crystal deposits in knee samples from arthritic patients, 2009, **1**, (2), 142-147 (Results published in this paper are included in Chapter 2).

2. Rawling T., C.E Austin, D. Hare, P.A. Doble, H.M. Zareie, and A.M. McDonagh, *Nano Research*, Thin Films of Ruthenium Phthalocyanine Complexes, 2009, **2**, (9), 678-687 (Results published in this paper are included in Chapter 4).

3. Austin C., D. Hare, T. Rawling, A.M. McDonagh and P. Doble, *Journal of Analytical Atomic Spectrometry*, Quantification method for elemental bio-imaging by LA-ICP-MS using metal spiked PMMA films, 2010, **25**, (5), 722-725 (Results published in this paper are included in Chapter 3).

4. Austin C., F. Fryer, J. Lear, D. Bishop, D. Hare, T. Rawling, L. Kirkup, A. McDonagh and P. Doble, *Journal of Analytical Atomic Spectrometry,* Factors affecting internal standard selection for quantitative elemental bio-imaging of soft tissues by LA-ICP-MS, 2011, **26**, (7), 1494-1501 (Results submitted in this paper are included in Chapter 4).

5. Austin C., Hare D., Rozelle A.L., Robinson W.H., Grimm R. and Doble P., *International Symposium on Metallomics 2009*, Cincinnati, USA, Elemental bio-imaging of calcium phosphate based crystal deposits in knee samples from arthritic patients. Oral presentation.

6. Austin C., Rawling T., Hare D., McDonagh A., Doble P., *International Symposium on Metallomics 2009*, Cincinnati, USA, Quantification method for elemental bio-imaging by LA ICP-MS using spiked thin films. Oral presentation.

Abbreviations

AFM	Atomic force microscopy
ARS	Alizarin red S
BCP	Basic calcium phosphate
CaP	Calcium phosphate-based
CF	Concentration factor
CPPD	Calcium pyrophosphate dihydrate
CPLM	Compensated polarised light microscopy
CPM	Chemical phase mapping
CPMM	Calcium phosphate monobasic monohydrate
CPP	Calcium pyrophosphate
cps	Counts per second
CRC	Collision/reaction cell
CRM	Certified reference material
csv	Comma separated value
DRC	Dynamic reaction cell
DMF	N,N-Dimethylformamide
DMSO	Dimethyl sulfoxide
EHDP	^{14}C-ethane-1-hydroxy-1,1-disphonate
EDS	Energy dispersive spectrometer
EDTA	Ethylenediaminetetraaccetic acid
EMPA	Electron microprobe analysis
ESEM	Environmental scanning electron microscope
ETV	Electrothermal vaporisation
FAA	Flame atomic absorption
FI	Flow injection
FIP	First ionisation potential
FSXRM	Full spectrum X-ray mapping
FTIRM	Fourier transform infrared microscopy
HA	Hydroxyapatite
HPLC	High pressure liquid chromatography
ICP-MS	Inductively coupled plasma-mass spectrometry
i.d.	Internal diameter

IDMS	Isotope dilution mass spectrometry
IS	Internal standard
ISF	Internal standard film
KED	Kinetic energy discrimination
LA	Laser ablation
LFC	Large format cell
LOD	Limit of detection
LOQ	Limit of quantification
MALDI	Matrix assisted laser desorption/ionisation
MRI	Magnetic resonance imaging
MSU	Monosodium urate
m/z	Mass-to-charge ratio
Nd:YAG	Neodymium doped yttrium aluminium garnet
NIST	National Institute of Standards and technology
OA	Osteoarthritis
PA	Pulse/analogue
PAA	Poly(acrylic acid)
PAGE	Polyacrylamide gel electrophoresis
P/B	Peak-to-background ratio
PEI	Poly(ethyleneimine)
PEO	poly(ethylene oxide)
PhCl	Chlorobenzene
PIXE	Particle induced X-ray emission
PLM	Polarised light microscopy
PMMA	Poly(methylmethacrylate)
PsA	Psoriatic arthritis
PVA	Poly(vinyl alcohol)
qMS	Quadrupole mass spectrometer
QUT	Queensland University of Technology
QXRM	Quantitative X-ray mapping
RA	Rheumatoid arthritis
RBS	Rutherford backscattering spectrometry
REE	Rare earth element
RGB	Red, green, blue

ROI	Region of interest
RSD	Relative standard deviation
RSF	Relative sensitivity factor
RT	Room temperature
RuPc	Ruthenium phthalocyanine
SAM	Self assembled monolayer
S/B	Signal-to-background ratio
SEM	Scanning electron microscopy
SFMS	Sector field mass spectrometry
SIMS	Secondary ion mass spectrometry
SN	Solution nebulisation
STEM	Scanning transmission electron microscopy
SXRF	Synchrotron-based X-ray fluorescence
TDS	Total dissolved solids
TEM	Transmission electron microscopy
TOF	Time of flight
W-10 %	Peak width at 10 % of the peak height
WDS	Wave dispersive spectrometer
XAS	X-ray absorption spectroscopy
XRD	X-ray diffraction
XRM	X-ray mapping
ZAF	Atomic number, absorption and fluorescence

Abstract

This thesis investigates an elemental bio-imaging technique that may be used to detect calcium phosphate-based (CaP) crystals in cartilage. CaP crystals are associated with osteoarthritis and define a subset of other arthritides. It is not yet known if these crystals play a direct role in disease conception/progression or are markers of joint damage. Improving our understanding of the processes involved in crystal formation and their relationship to arthritis may lead to the identification of therapy targets or biomarkers, enabling the development of effective treatments or early detection and monitoring of the disease. This is hindered by the small size and complex biological matrix which make crystal detection difficult using traditional technologies. Greater understanding may be achieved through the application of novel technologies, such as those described in this thesis, to crystal detection in biological materials.

Metallomics is an emerging field first defined in the early 2000's. It is the study of free metals and metal containing species; their interactions, transformations and functions in biological systems. The study of metals is of great importance since many metals play essential roles in maintaining physiological functions or cause toxicity in organisms. Spatially resolved elemental maps offer unique insights into the role of metals at the tissue and cellular level. The production of element distribution maps is termed elemental bio-imaging.

Laser ablation–inductively coupled plasma–mass spectrometry (LA-ICP-MS) is an elemental bio-imaging technique capable of providing elemental maps, increasingly applied to the study of metals and non-metals in biological samples. LA-ICP-MS offers the benefits of direct multi-element analysis of solid samples with minimal sample preparation, high sensitivity and detection of trace, minor and major elements. In this study LA-ICP-MS was applied to the detection and identification of calcium phosphate-based (CaP) crystals in human cartilage and synovial fluid samples.

The LA-ICP-MS elemental bio-imaging method was developed to detect and identify CaP crystals in cartilage and synovial fluid. The analysis of Ca is hindered by interfering species in the mass spectrum (e.g. $^{40}Ar^{+}$, $^{12}C^{16}O_2^{+}$). Two methods of interference reduction were investigated to improve Ca detection: cool plasma and collision/reaction cell (CRC). The CRC method gave the best improvements in signal-to-background ratios, detection limits and

isotope ratio accuracy. The affect of Ca ($^{44}Ca_2^+$) and Sr-based ($^{88}Sr^{2+}$) interferences on Sr and Ca isotope signal intensities was also investigated. Both elements produced a negligible effect on the respective analyte signal intensities.

Development of a new quantification procedure was undertaken to further improve the LA-ICP-MS imaging method by defining a scale for easy crystal detection. Current quantification procedures are time consuming and laborious. Thin polymer films spiked with analytes and prepared by the spin coating technique were validated using tissue standards and finally used to quantify cartilage sections stained for CaP crystals. The films were prepared from a solution containing 10 % PMMA, 40 % xylene and 50 % chlorobenzene. The new quantification procedure also enabled the inclusion of multiple internal standards (IS) by placing the tissue sample on top of the film. Factors affecting the efficacy of ISs were also investigated. A close match in mass was the dominating factor in selecting optimal analyte/IS pairings and ablation cell design was also identified as an important factor in IS selection. For soft tissue analysis, ^{13}C was found to be an effective IS but an element closer in mass to the analyte provided better signal compensation.

The developed LA-ICP-MS elemental bio-imaging technique was successfully applied to the detection of CaP crystals in cartilage. This is the first study to show the correlation between CaP crystals and Sr and therefore this technique may provide new insights into the processes involved in crystal generation and their relationship to arthritis.